Steroid hormones: **Interactions with membrane-bound receptors**

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Steroid hormones are generally thought to pass easily across a plasma membrane into a cell, interacting once inside with soluble nuclear receptors, but recent experiments have demonstrated the importance of membrane-bound receptors in mediating the activity and the metabolism of steroid hormones.

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Hormones traditionally have been classified into two major groups: hydrophilic peptides or amino acid derivatives, which exert their actions via membrane-bound receptors; and hydrophobic, cholesterol-derived steroids, which diffuse across the cellular membrane and act on receptors at the nuclear level. Such categorization, however, is likely to be an oversimplification. Recent studies suggest that steroid hormones use receptors on cellular membranes both to gain access to the intracellular compartment and to modulate cellular functions. These interactions with cell-surface receptors have important physiological consequences.

The classical model of steroid hormone action

Clever and Karlson [1] first showed that steroid hormones act at the genomic level in 1960, when they showed that ecdysone can induce 'puffs' on giant insect chromosomes. Subsequent observations revealed that a fundamental action of steroid hormones — the induction of protein synthesis — correlated with apparent changes in gene transcription (reviewed in [2]). The implication was that the chromosome puffs are formed as a consequence of the transcriptional activation of specific genes. Intracellular receptors for steroid hormones were subsequently identified, beginning in the 1960s, and genes encoding many of them were cloned in the 1980s and 1990s.

These discoveries were the origins of the classical model for steroid hormone action (Figure 1a). In this model, the hydrophobic steroid hormones are transported in the circulation largely bound to plasma carrier proteins. The bound hormones are biologically inactive. According to the 'free hormone hypothesis' (reviewed in [3]), steroid hormones exert their effects after dissociating from the carrier proteins. It is thought that, because of their lipophilic nature, free steroid hormones enter target cells primarily by passive diffusion through the cell membrane. After gaining entry to the cell, steroid hormones act as ligands for nuclear receptors and alter gene transcription (reviewed in [4]).

A cell-surface receptor for a steroid hormone

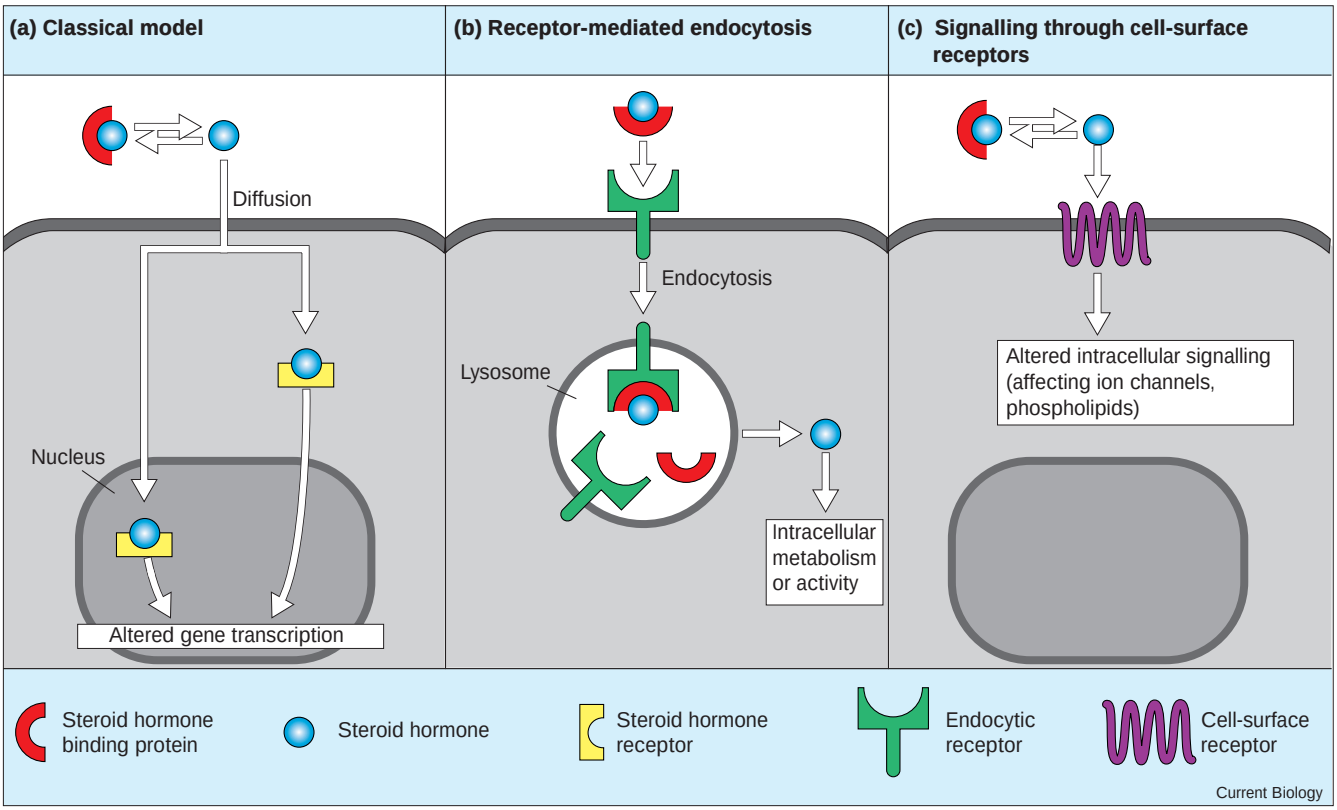
Passive diffusion of steroid hormones across cell membranes does not, however, adequately explain several observations regarding the cellular uptake of steroid hormones. Kinetic studies of steroid hormone metabolism, such as those for vitamin D, have suggested that the non-specific diffusion of free hormones across cell membranes cannot fully account for their clearance rates from the serum [5]. Furthermore, some steroid target tissues express binding sites for carrier proteins (reviewed in [6]), and certain human breast cancer cells can internalize testosterone/estradiol-binding protein by receptor-mediated endocytosis [7]. These findings have suggested that receptor-mediated uptake of steroid hormones, bound to their carrier proteins, may play a role in steroid metabolism, although the physiological significance of such pathways has been unclear.

A recent study by Willnow and colleagues [8] alters our view of steroid hormone metabolism by demonstrating both the existence and the biological importance of a receptor-mediated endocytosis pathway for a steroid-like hormone in its carrier-protein-bound state. These researchers studied 'knockout' mice lacking megalin, a membrane receptor that is a member of the low density lipoprotein (LDL) receptor family and is expressed on the apical surfaces of several epithelial cell types, including those in the choroid plexus and the proximal tubule of the kidney. Like several other members of the LDL receptor family, megalin has relatively broad ligand-binding specificity and mediates the cellular uptake of a number of macromolecules.

Although most megalin$^{-/-}$ mice die perinatally because of defective forebrain development [9], approximately one in fifty survive to adulthood. It was these mice, which exhibit severe bone malformation and dramatically reduced bone density, that Nykjaer *et al.* [8] studied. Because megalin had previously been shown to be capable of taking up molecules injected into proximal renal tubules, and because electron microscopy of kidneys from megalin$^{-/-}$ mice demonstrated fewer endocytic organelles than normal, the researchers hypothesized that megalin$^{-/-}$ mice may be unable to resorb certain macromolecules from the urine.

To test this hypothesis, Nykjaer *et al.* [8] performed a simple but key experiment — they compared the urinary

Figure 1



Three ways that a steroid hormone can interact with a cell. (a) The classical model. The steroid hormone dissociates from its plasma carrier protein and diffuses across the cell membrane. After gaining entry to the cell, the free hormone binds to an intracellular receptor and alters gene transcription. (b) Receptor-mediated endocytosis. The steroid hormone, bound to its plasma carrier protein, is brought into the cell via a cell-surface receptor. The complex is broken down inside the lysosome, and free steroid hormone diffuses into the cell, where it subsequently exerts its action at the genomic level or undergoes metabolism. (c) Signalling through cell-surface receptors. The free steroid hormone alters intracellular signalling by binding to cell-surface receptors. The steroid hormone could exert these effects directly or could alter signalling by blocking the actions of peptide hormones.

proteins of megalin$^{-/-}$ mice with those of wild-type mice by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. They found that several low-molecular-weight proteins were present only in the urine of the megalin$^{-/-}$ mice. One of these proteins was identified as vitamin D-binding protein, the principal carrier protein for vitamin D in the serum. The investigators went on to show that urinary excretion of vitamin D-binding protein and 25-(OH) vitamin D_3 was markedly increased in megalin$^{-/-}$ mice, and there was an accompanying 80% reduction of plasma 25-(OH) vitamin D_3. Moreover, similar changes could be induced in rats by infusing kidney tubules with receptor-associated protein, a protein that binds to megalin and other LDL-receptor family members and prevents them from binding ligands.

Megalin deficiency also decreased the conversion of 25-(OH) vitamin D_3 to its active metabolite, 1,25-$(OH)_2$ vitamin D_3, a process that is catalyzed by a mitochondrial hydroxylase in proximal tubular cells. This effect was also produced by infusion of receptor-associated protein in rats. Perfusing rats with tracer-labeled 25-(OH) vitamin D_3 bound to vitamin D-binding protein resulted in the recovery of tracer-labeled 1,25-$(OH)_2$ vitamin D_3 from the serum; in contrast, no tracer-labeled dihydroxylated products were detected in the plasma or the urine of animals co-infused with receptor-associated protein. Thus, the lack of megalin causes both decreased serum levels of 25-(OH) vitamin D_3 and deficiency of 1,25-$(OH)_2$ vitamin D_3 conversion. The resultant severe vitamin D deficiency apparently accounts for the skeletal deformities and decreased bone density in megalin$^{-/-}$ mice.

Taken together, these experiments show that vitamin D, a steroid-like hormone, is taken up in its vitamin D-binding protein-bound state into renal tubular cells via the endocytic receptor megalin, and that the receptor is necessary to maintain normal vitamin D homeostasis. Without this mechanism, there are defects in both the storage and the activation of the hormone. Thus, these

studies demonstrate that a receptor-mediated endocytosis pathway not only exists for a steroid-like hormone but also plays a crucial role in the hormone's activity and metabolism (Figure 1b).

Membrane receptors in steroid hormone action

Evidence is accumulating that cell-membrane receptors can not only take up steroid hormones through endocytosis, but also mediate steroid hormone action directly in some cases. Steroid hormones sometimes elicit extremely fast cellular responses (within seconds to minutes), which are too rapid to be attributed to induced RNA or protein synthesis. In the late 1960s, Szego *et al.* [10] showed that estrogen acutely raised cyclic AMP levels in rat uterus; several years later, Pietras *et al.* [11] identified specific binding sites for estrogen at the outer surfaces of isolated endometrial cells. Since then, steroid hormone action has been implicated in the opening and closing ion channels, the generation of second messengers, and the phosphorylation of transcription factors (reviewed in [12]), all independent of any action at the genomic level.

How do steroid hormones act at the cell membrane? One way seems to involve direct binding of steroids to receptors of peptide hormones. Grazzini *et al.* [13] have recently elucidated the mechanism by which progesterone, a steroid hormone essential for maintaining mammalian pregnancy, can effect uterine quiescence without changing gene expression or protein synthesis. They found that progesterone directly inhibited the activity of oxytocin, a peptide capable of inducing uterine contractions, by binding to cell-surface oxytocin receptors. This action of progesterone reduced two functional consequences of oxytocin signalling: production of the second messenger inositol-1,4,5-triphosphate and an increase in intracellular calcium concentration. These results therefore illustrate another mechanism of steroid hormone action — the modulation of signalling through cell-surface receptors (Figure 1c). In some instances, steroid hormones might also bind specifically to cell-surface steroid hormone receptors [14].

Why are there multiple pathways of steroid action?

Why should steroid hormones interact with receptors both at the cell surface and in the nucleus? Szego [15] has suggested that the synthesis of macromolecules as a result of genomic actions of steroid hormones must be preceded by rapid changes in the cellular environment to 'prime' the cell and support such activity. These preparative changes may include altered ion influxes, import of amino acids and sugars, or phosphorylation of key enzymes, all of which could be triggered by interactions of steroid hormones with cell-surface receptors.

The interaction of steroid hormones with endocytic receptors could serve to target steroids to specific cell types in order to augment their effects or metabolism. In contrast to the classical model, in which steroid hormones diffuse into target tissues in a non-specific fashion, receptor-mediated endocytosis could serve to concentrate steroid hormones in particular cell types, thereby increasing hormonal activity at the target tissues without exposing the rest of the organism to equally high levels of the hormone. Alternatively, as shown for vitamin D and megalin, receptor-mediated endocytosis of the carrier protein–steroid hormone complex might target a hormone to a specific cell type that is important for its metabolism.

Perspectives

Recent advances have demonstrated the importance of membrane-bound receptors in mediating the activity and the metabolism of steroid hormones. These new discoveries raise a number of questions. Are the cases discovered above isolated findings, or are many steroid hormones capable of interacting with cell-surface receptors? If so, which steroids and receptors are involved, and what roles do these interactions play in their actions or metabolism? These advances may also have important medical implications. For example, these receptor–steroid interactions might provide new sites for therapeutic interventions: perhaps a compound that solely antagonizes the receptor-mediated uptake of bound steroid hormones would be more efficacious or cause fewer side effects than a non-selective steroid antagonist. Functional studies of specific receptors may provide answers to these questions.

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